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Review

Fungi associated with Esca disease of grapevine in Germany

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Summary

Esca disease of grapevine is gaining increasing importance in Central European wine-growing countries. Several fungi, all of which are wood-inhabiting, were found to be associated with the disease. The taxa thought to act as main causal agents are the basidiomycete, *Fomitiporia mediterranea*, and, less frequently, the deuteromycetes, *Phaeomoniella chlamydospora* and *Phaeoacremonium aleophilum*. In addition, the species *Eutypa lata*, *Phomopsis viticola*, *Botryosphaeria obtusa*, and *Cylindrocarpon destructans* were isolated from Esca-affected vines. These species have been described in a standardized style and information is provided on taxonomy, cultured mycelium, microscopical characters, nuclear behaviour, as well as restriction and sequence data of ribosomal DNA.

K e y w o r d s : Esca, fungi, *Fomitiporia mediterranea*, *Phaeomoniella chlamydospora*, *Phaeoacremonium aleophilum*.

Introduction

Esca is a widespread disease of grapevine in many countries all over the world (overview: CHIARAPPA 2000). It is generally accepted that Esca is not a new disease; in fact, it was recognized already more than 100 years ago in the mediterranean region as well as in California (RAVAZ 1898, PETRI 1912, VIALA 1926, BOURDOT and GALZIN 1927). There is even some evidence that the disease may be as old as vine cultivation itself, since references to esca-like symptoms were found in several ancient Greek and Latin works (VIALA 1926, MUGNAI *et al*. 1999, SURICO 2000).

A dramatic upsurge of the disease has been reported in recent years. This is most obvious for the mediterranean vine-growing countries, where in some areas such as Tuscany more than 50 % of the vineyards have a disease incidence ranging from 20 to 30 % (CORTESI *et al*. 2000 a). The average annual increase is estimated to be 4-5 % (MUGNAI *et al*. 1999). Similar observations have been made for France, Greece, or Portugal (MUGNAI *et al*. 1999, REGO *et al.* 2000, ARMENGOL *et al*. 2001, REDONDO *et al.* 2001, RUMBOS and RUMBAU 2001). It is striking to note that also the geographical range of the disease has extended; for instance, the vine-growing regions of Germany and Austria, essentially not affected by the disease up to the nineties, reported increasing incidence of Esca over the last few years (KASSEMEYER 1998, REISENZEIN *et al*. 2000, FISCHER and KASSEMEYER 2002, KASSEMEYER *et al.* 2002). In Germany, Esca has been shown to exist since approximately 15 years at least (FISCHER and KASSEMEYER 2002). Presently, the most severely affected areas are located in the southwestern part.

Esca is a complex disease comprising an array of symptoms. Usually within several years ("chronic esca"), but also within several months only ("acute esca") vines are killed by the disease. Economic losses can be considerable, and any control of the disease by means of chemicals and/or based on altered viticultural management seems unlikely for the near future. In spite of intensive studies, the etiology of the disease is still not fully understood. It is therefore a main goal of these studies to unequivocally identify the causal agents and to learn as much as possible about their biology and life strategies. These data shall be used as a basis for far-reaching strategies in order to control the disease.

Esca comprises symptoms inside the trunk and larger branches, on the shoots, on the leaves and on the berries. While the symptoms on leaves and berries can vary considerably from year to year, even for the same vines (MUGNAI *et al.* 1999), wood decay symptoms are relatively stable. Thus it is concluded that its appearance in the woody parts provides the most reliable information on the occurrence and, to a certain degree, the intensity of the disease.

Symptoms in the wood such as white rot or small, dark-brown or black spots in cross sections indicate wood-inhabiting fungi to act as a potential pathogenic source of the Esca disease. It may be speculated that the symptoms on leaves and/or berries are caused by extracellular fungal toxins segregated into the vessels of the plant. In fact, the chemical structure of such toxins has been partly clarified in relation to other wood affecting diseases of grapevine (SPARAPANO *et al*. 2000, 2001, TABACCHI *et al*. 2000).

In 2001, a specific project was initiated by the State Institute for Viticulture in Freiburg concentrating on several aspects of Esca; among other things, emphasis was given to the putative organisms involved in the disease. In the course of investigations a considerable number of Esca-affected vines was examined and the spectrum of wood-colonizing fungi was elucidated. Vines were different in age and geographic origin, and represented most of the cultivars grown in Germany.

In this paper, the fungi most frequently isolated from the infected vines are presented both by illustrations and text. In this way, the morphology and microscopy of cultured mycelium, the nuclear behaviour of conidia and vegetative hyphae, and restriction and sequence data of the ribosomal ITS region are provided for the species *Fomitiporia mediterranea*, *Phaeomoniella chlamydospora*,

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Phaeoacremonium aleophilum, *Eutypa lata*, *Phomopsis viticola*, *Botryosphaeria obtusa*, and *Cylindrocarpon destructans*. Although far from being complete, this compilation may provide a sound basis for a reliable assignment of the organisms involved in Esca disease in Germany.

In the literature, dispersed information is found in relation to lignicolous fungi and their possible occurrence on vine. These data have been compiled in an appendix and have been supplemented with our own observations. It should be noted that none of the enlisted fungi plays a role in Esca; nevertheless, they often indicate a somewhat weakened condition of the plant. The respective fruitbodies, which in most cases are formed regularly on the vine, are noticeable also for the less trained eye.

Material and Methods

Sampling of vines: The plants studied originated from different parts of Germany, mostly Baden-Württemberg, but also Rhineland-Palatinate, Hesse, Bavaria, and Saxony. Age of plants ranged from 1 to approximately 40 years. Esca-affected plants included red wine cultivars such as Merlot, Pinot meunier, Pinot noir, and Trollinger, and white wine cultivars such as Bacchus, Chasselas, Chardonnay, Pinot blanc, Pinot gris, Kerner, Morio-Muskat, Müller-Thurgau, Muskateller, Riesling, Ruländer, Scheurebe, Silvaner, and Traminer.

Isolation and culturing of fungi associated with esca symptoms: The trunks of diseased vines were cut into 3-5 parts of equal length. Exfoliating bark was peeled off and mycelia were isolated from symptomatic wood. Each section of the trunk was surface-sterilized by submersion in 30 % H_2O_2 for 30 s, followed by flaming. Using a sterile scalpel, a thin slice of the outer wood was removed from the surface, and small wood chips were sliced along the necrotic and/or spongy wood areas across the dark-brown or discolored line between healthy and diseased wood. Three chips were placed into 9 cm diameter petri plates containing ME (malt extract medium; 2 % agar, 2 % malt extract, 0.05 % yeast extract) or PDA (potato dextrose agar; 1.5 % agar, 0.4 % potato extract, 2 % glucose). Petri plates were incubated under permanent dark conditions at 21 °C.

For determination of mycelial growth rates selected strains were incubated on ME at 21 °C under permanent dark conditions. Mycelial growth was measured by calculating the mean of two perpendicular colony diameters. Two repeats were performed for each isolate.

Comparative microscopy: Usually slide cultures (VAN UDEN 1951) were used for comparative microscopy of vegetative cultures. Observations were made in a drop of water, Melzer´s reagent, or lactophenol-cotton blue (MEIXNER 1975) at 500x or 1250x using phase contrast optics. Twenty observations were recorded for measurements of hyphae and conidia. Nuclei of conidia or mycelium were stained with Giemsa (FISCHER 1987) for light microscopy, and with DAPI for fluorescence microscopy (MEIXNER and BRESINSKY 1988). Micrographs were obtained under differential interference contrast optics (ZEISS Axiophot equipped with the digital camera Axiocam and the imaging software Axiovision).

DNA isolation and PCR amplification: Whole cell DNA was isolated from fresh mycelium; isolation was essentially as described by LEE and TAYLOR (1990). Quantity and quality of the DNA were examined on 1 % agarose gels. Isolated DNA was diluted 1:100 in distilled water. The polymerase chain reaction (PCR) was used to amplify a portion of the nuclear encoded ribosomal DNA unit defined by the primer combination prITS5 and prITS4 (for primer sequences, see WHITE *et al*. 1990). The fragment spans the entire ITS1 region, the 5.8S rRNA gene, and the ITS2 region.

The PCR reactions were set up in 50 µl volumes and were overlayed with two drops of mineral oil. Hot start PCR was applied throughout (D'AQUILA *et al*. 1991). Forty cycles were performed on a TRIO-Thermoblock (Biometra, Germany), using the following parameters: 95 °C denaturation step (1 min), 50 °C annealing step (1 min), 72 °C primer extension (1 min). A final incubation step at 72 °C (7 min) was added after the final cycle. 5 µl of each PCR reaction were electrophoresed on 1 % agarose gels. DNA molecular weight marker VI (Roche Diagnostics, Germany) was used as standard. The amplified products were purified with the QIAquick PCR Purification Kit (Qiagen, Germany) following the manufacturer's instructions. DNA was suspended in 50 µl Tris-HCl buffer (10 mM, pH 8.0).

Restriction analyses: For restriction analysis, PCR products were extracted with one volume of 1:1 phenol/chloroform and centrifuged at 10.000 x *g* for 15 min; 80 µl of the aqueous portion were removed, and DNA was precipitated by the addition of 8 µl of sodium acetate (pH 8.0) and 190 µl of 100% ethanol (> 1 h, -20° C). Precipitates were collected by centrifugation (10.000 x *g*, 15 min), washed with 750 µl of 70 % ethanol, and resuspended in 30-50 µl TE buffer. For restriction analysis, the restriction enzymes *Hpa* II and *Mbo* I were used according to the manufacturer´s instructions (MBI Fermentas, Vilnius, Lithuania). The restriction products were separated on 2.5 % agarose gels. Fragment lengths were calculated using the Webcutter 2.0 program.

Sequencing: Representative strains of the isolated fungi were included in the sequencing experiments. Instead of mycelium derived from diseased wood, single spore isolates were used for strains of *Fomitiporia mediterranea*. Fragments were sequenced with the AmpliTaq DNA Polymerase FS Dye Terminator Cycle Sequencing kit (Perkin Elmer, USA), using 2 µl of premix, 1 µl of the primers (8 pmol of prITS1 and prITS4, respectively), and 3.5 µl of the PCR products. The reactions were set up in 11 µl volumes, and were overlayed with one drop of mineral oil.

Sequences were generated in two directions and 25 amplification cycles were carried out, using the following parameters: 96 °C denaturation step (30 s), 59 °C annealing step (15 s) for prITS1, 53 °C annealing step (15 s) for prITS4, 60 °C primer extension (4 min). DNA was precipitated by addition of 2 µl of NaAc (3 M, pH 4.8) and 55 µl of EtOH 100 %, and was then washed with 150 µl of EtOH 70 %. The DNA pellet was resuspended in 1:4 EDTA (50 mM, pH 8.0): formamide.

The electrophoresis was done with an ABI 373A Automatic Sequencer (Perkin Elmer). After processing the raw data with SeqEd (version 3.0), conspecific sequences were aligned using the ClustalX (version 1.64b) program (THOMPSON *et al*. 1997) and, when possible, were compared with respective sequences deposited in GenBank.

Results and Discussion

Sampling of infected vines: All in all, 156 esca-affected vines were sampled in 2001 and 2002; distribution of the isolated fungal organisms is given in Tab. 1.

The basidiomycete, *Fomitiporia mediterranea* (*Fmed*), described only recently (FISCHER 2002), was found to be the predominating fungus, and was isolated out of 63 % of the sampled vines. *Fmed* causes a white rot, and usually was recovered from zones of wood decay. While white rot was most evident in the uppermost part of the trunk, next to the pruning wounds, it also was found to extend into the more basal parts of the plant; here, it was often limited to the area around the pith or it spreaded along a sector, eventually reaching the surface of the trunk. In several cases *Fmed* was isolated out of darkened, very hard wood, colonized by the ascomycete, *Eutypa lata*. Remarkably, this species was frequently isolated from Esca-diseased vines (26 %), and often occurred side-by-side with *Fmed* (22 %).

Table 1

Fungal organisms in esca-affected grapevines (n=156)

Fungal organisms	Frequency (%)
Fomitiporia mediterranea (*Fmed*)	98 (63 %)
Phaeomoniella chlamydospora (*Pch*)	
Phaeoacremonium aleophilum (*Pal*)	46 (30 %)
Eutypa lata	40 (26 %)
Others[1]	70 (45 %)
Fmed + *Pch/Pal*	12 (8 %)
Fmed + *Eutypa lata*	32 (22 %)
Fmed + others[1)]	38 (24 %)
Pch/Pal + *Eutypa lata*	8 (5 %)
Pch/Pal + others[1]	10 (6 %)

[1)] mostly *Botryosphaeria*, *Cylindrocarpon*, *Phomopsis*; also unidentified mycelia.

Fungi assignable to the genera *Phaeomoniella* and *Phaeoacremonium* were isolated less frequently (30 %). Usually these organisms were associated with small, brown or black spots distributed around an annual growth ring or they were recovered from the woody tissues close to the pith. The dark spots, appearing as dark streaks in longitudinal sections, and often associated with the presence of gummy masses in the xylem vessels, in several cases were found to extend into the roots. The mycelial cultures isolated from such discoloured wood were mostly identified as *Phaeomoniella chlamydospora* (*Pch*) and, sometimes, *Phaeoacremonium aleophilum* (*Pal*); distinction to related taxa was often difficult in the latter case (see notes below). Sometimes, *Pch* and *Pal* could also be isolated from wood that had been decayed by white rot, and together with *Fmed* they grew out from the same wood chip in culture. In general, however, joint occurrence of *Fmed* and *Pch* respectively *Pal* in the same vine was observed less frequently (8 %).

45 % of the sampled vines were infected by some other ascomycetous fungi such as *Botryosphaeria obtusa*, *Cylindrocarpon destructans*, or *Phomopsis viticola*. While identification was readily accomplished for the two latter taxa, not all of the *Botryosphaeria* isolates could unequivocally be assigned to *B. obtusa*. None of these three taxa is thought to be closely associated with Esca, instead they have their own symptomatology (MUGNAI *et al*. 1999). These data demonstrate that different diseases may occur side-by-side in the same vine, in this way probably promoting the diseases´ progress.

The symptoms of wood deterioration caused by *Pch* and species of *Phaeoacremonium* became visible together with or, mostly, were preceding the white rot symptoms. The systematic isolation of fungi from discoloured wood indicate a close relation between individual stages of wood deterioration and particular fungal taxa. Based on the presented data, wood decay caused by *Fmed* seems to be the main reason for Esca disease in the geographic area under study, but quite often is preceded by discoloration of the wood caused by *Pch* and *Pal*. In this connexion it should be stated that *Pch* and *Pal* were isolated from plants between 1 year and 32 years old, while *Fmed* was isolated from plants between 4 years and approximately 40 years old.

Descriptions of fungal taxa

1. *Fomitiporia mediterranea* (M. Fischer): Taxonomy: Member in Hymenochaetaceae, Hymenochaetales, Basidiomycetes.

Cultured mycelium: Variable, two types can be distinguished at 21 °C on ME; type B ("bleaching type"): cottony to woolly, aerial hyphae yellowish to brownish, pigmentation of the medium weak or lacking, growth rate 3.0-4.5 cm in 14 d; type S ("staining type"): sparse development of aerial hyphae, pigmentation of the medium modest to strong, growth rate 1.5-2.5 cm in 14 d.

Microscopy (Fig.1): Hyphae septate and branched, hyaline to yellowish brown, smooth, septa partially hardly visible, without clamps, 1.5-5.5 µm wide; conidia absent; side-branches often arising next to septum.

Nuclear behaviour: Hyphal segments oligokaryotic, 2-4 (6-8) nuclei; hyphal tips often with distinctly increased number of nuclei. ITS fragments (strain 45/23.3): *Hpa* II: no restriction sites; *Mbo* I: 3 fragments (3, 316, 425 bp). ITS sequence (strain 45/23.3): 744 bp long.

AAGGATCATTAACGAGTTGGAACGTGGAGGTTGATGC
TGGTGCATATATAGTGTACATGTGTGCTCGCCTTCACA
CTCTTCATCCACTCAACCCCTGTGCACTTTATCAGAGTT
AGTAATAGTATTGTGGTGGCAGCCGTTTGTTATTCATTG
TTAGAAGCGGGGTAACTACTTTCTAGCAGTAGTAATAAT
AACAATCTTGGTTCTACTACTATTACTGTGAACACTTTGA
CTTTTACTTATACAAACACTTTGCTTGTTCTTGTGAATGTG
TAATGCTCCTTGTGAGCGAAATACAAATATACAACTTTCA
ACAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAG

CGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAA
TCATCGAATCTTTGAACGCACCTTGCGCCCCTTGGTATTCC
GAGGGGCATGCCTGTTTGAGTGTCATGTAATTCTCAATCCT
CTTTTTTCTTAATTGAAGAGGGGGCTTGGACTTGGAGGTTA
ATATATATACATGCTGGTACTGTCTGTATCGGCTCCTCTAA
AATGCATTAGCTGGACTGTAGTTCGCATTGTTTGGTGTAGT
AATAGTTTTCTATCTATATTCACTACAGTGCTTACTTAGACT
GTCTGCTTCTAATAGTCTGCCTATATGTCGGACAGGTACTCT
GTTACCTTAAACCATTTGACTCCTTTGACCTCAAATCAGGTA
GGTCTACCCGCTGAACTTAA

Notes: *Fomitiporia mediterranea* (*Fmed*) seems to be the main causal agent for Esca disease in Germany; data are somewhat less conclusive for other grape-growing countries. The species causes a white rot in the trunk; especially in the uppermost parts, less distinct in the basis. Fruit bodies usually are developed on dead trunks of *Vitis*. While *Fmed* is restricted to *Vitis* in Germany, it occurs on some other hardwood species as well in the Mediterranean region. For a long time *Fmed* has been misidentified as *Phellinus igniarius*, and, afterwards, as *Fomitiporia punctata*. Molecular and genetic data are necessary to distinguish between *F. punctata* and *Fmed*. Additional literature: FISCHER (2002).

2. *Phaeomoniella chlamydospora* (W. Gams, Crous, M. J. Wingf. & L. Mugnai) Crous & W. Gams: Taxonomy: Anamorphic member of the Herpotrichiellaceae, Chaetotyriales, Ascomycetes; teleomorph unknown.

Cultured mycelium: Colonies appressed, with sparse development of aerial hyphae, grey-olivaceous to olivaceous-black, pigmentation of the medium concolorous, growth rate 1.4-2.2 cm in 14 d.

Microscopy (Fig. 2): Hyphae septate and branched, occurring in strands of up to 8, green to brownish, smooth or with tiny warts, without clamps, (1.5) 2.0-5.0 µm wide; conidia common, smooth, forming slimy heads at the apices of conidiogenous cells, more or less hyaline, oblong-ellipsoidal - ovoid, straight, 1.5-5.0 x 1.0-2.0 µm; chlamydospores rare or common, globose to subglobose, mostly singular, but also in chains of up to 7, smooth or with tiny warts, 5.0-15.0 x 4.0-15.0 µm.

Nuclear behaviour: Hyphal segments of mycelium uni- or binucleate; conidia uninucleate; chlamydospores with up to 7 nuclei. ITS fragments (strain MT.Zi.12): *Hpa* II: 2 fragments (180, 370 bp); *Mbo* I: 5 fragments (7, 17, 31, 175, 320 bp). ITS sequence (strain MT.Zi.12): 550 bp long.

AAGGATCATTATCGAGTCAGGGTCCTCTGGGCCCGAT
CTCCAACCCTTTGTTTATCATACCTTTGTTGCTTTGGC
AGACCCGTCCTTCGGGACCGTCGGGGGCGTTCAGTC
GCCTCTGGCCAGCGTCTGCCAGTAGCCCAACCAAAA
TTCTTTGTTACATGTGACGTCTGAACGGTTCCATCAA
AATCAAACCAAAACTTTCAACAACGGATCTCTTGGTT
CTGGCATCGATGAAGAACGCAGCGAAATGCGATAAG
TAATGTGAATTGCAGAATTCAGTGAATCATCGAATCT
TTGAACGCACATTGCGCCCTTTGGTATTCCGAAGGGC
ATGCCTGTTCGAGCGTCATTATCAACCCTCAAGCCCG
GCTTGATATTGGGTCCATATCAACTTTCATAGAAGAT
AGGCCCGAAAGATAATGGCGGCGTCAAGAATGACCC
CAGGTGCAGCGAGCAATCAAGCATACACTGAGGTGG
TCCTCTTGGCCTGGCCCTATTGTTTTGTTGCAGAACTC
TCAGGTTGACCTCGGATCAGGTAGGAATACC

Notes: *Phaeomoniella chlamydospora* (*Pch*) is usually present in wood showing stages of discoloration such as "brown wood-streaking" or black streaks; it is often associated with black exudate that oozes from the xylem when vines are cut in cross section. However, the fungus is difficult to isolate because of slow growth of colonies, which may require several weeks before growing out from wood chips onto agar plates. *Pch* together with *Phaeoacremonium aleophilum* (*Pal*; see below) is discussed as the main causal agent of Esca ("Petri disease") in some grape-growing countries. More literature, providing data on morphology, microscopy, and the molecular background: CROUS *et al.* (1996), CROUS and GAMS (2000), and GROENEWALD *et al.* (2001).

3. *Phaeoacremonium aleophilum* (W. Gams, Crous, M. J. Wingfield & L. Mugnai): Taxonomy: Anamorphic member of the Herpotrichiellaceae, Chaetotyriales, Ascomycetes; teleomorph *Togninia minima* (Tulasne & C. Tulasne) Berlese.

Cultured mycelium: Colonies wooly, with weak development of aerial hyphae, honey to olivaceous-brown, pigmentation of the medium concolorous, growth rate 1.0-1.6 cm in 14 d.

Microscopy (Fig. 3): Hyphae septate and branched, occurring in strands of up to 10, light brown, smooth or with tiny warts, without clamps, 1.5-4.0 µm wide; conidia common, smooth, forming slimy heads at the apices of the conidiogenous cells, hyaline, oblong ellipsoidal - allantoid, 3.0-6.0 x 1.5-2.5 µm; chlamydospores absent.

Nuclear behaviour: Hyphal segments of mycelium mostly uninucleate, rarely binucleate; conidia uninucleate. ITS fragments (strain 3302/I): *Hpa* II: 4 fragments (12, 25, 92, 426 bp); *Mbo* I: 4 fragments (3, 29, 203, 320 bp). ITS sequence (strain 3302/I): 555 bp long.

AGGGATCATTATCGAGTTTCGTACTCCAAACCCTTTG
TGAACATACCTGTTTTCGTTGCTTCGGCAGGTCGGG
GGCCAACCCCGCCCGCCGCCGGACTCCCCCTCGCG
GGGCTGCGCCGGCGGGCCTGCCGGAGGGCACAGAC
TCTGTATTCAAAAACGTACCTCTCTGAGTTATCTTTACA
AATAAGTAAAAACTTTCAACAACGGATCTCTTGGTTCTG
GCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATG
TGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACG
CACATTGCGCCCGCTAGTATTCTGGCGGGCATGCCTGT
CCGAGCGTCATTTCAACCCTCAGGCCCTGGTTGCCTGG
TGTTGGGGCGCCGCGTCCCCTCAGCGGGCGCGGGCC
CCGAAAGTCAGTGGCGGGCTCGCCAGGACTCCGAGCG
CAGTAGTTTACACCTCGCTGCGGAGGACCTGGCGGGTT
ACCCAGCCGTAAAACACCCCAAACTTCTAAGGTTGACCT
CGGATCAGGTAGGAATACCCGCTGAACTTAA

Notes: *Phaeoacremonium aleophilum* (*Pal*) can be distinguished from *Phaeomoniella chlamydospora* (*Pch*) by the strict absence of chlamydospores. Like *Pch*, the species is usually present in discoloured wood, but clearly was observed less often in our study. *Pal* is difficult to isolate as well; sometimes it was found together with *Pch*. More taxa of *Phaeoacremonium* are discussed to be related to Esca symptoms, such as *P. angustius*, *P. inflatipes*, and

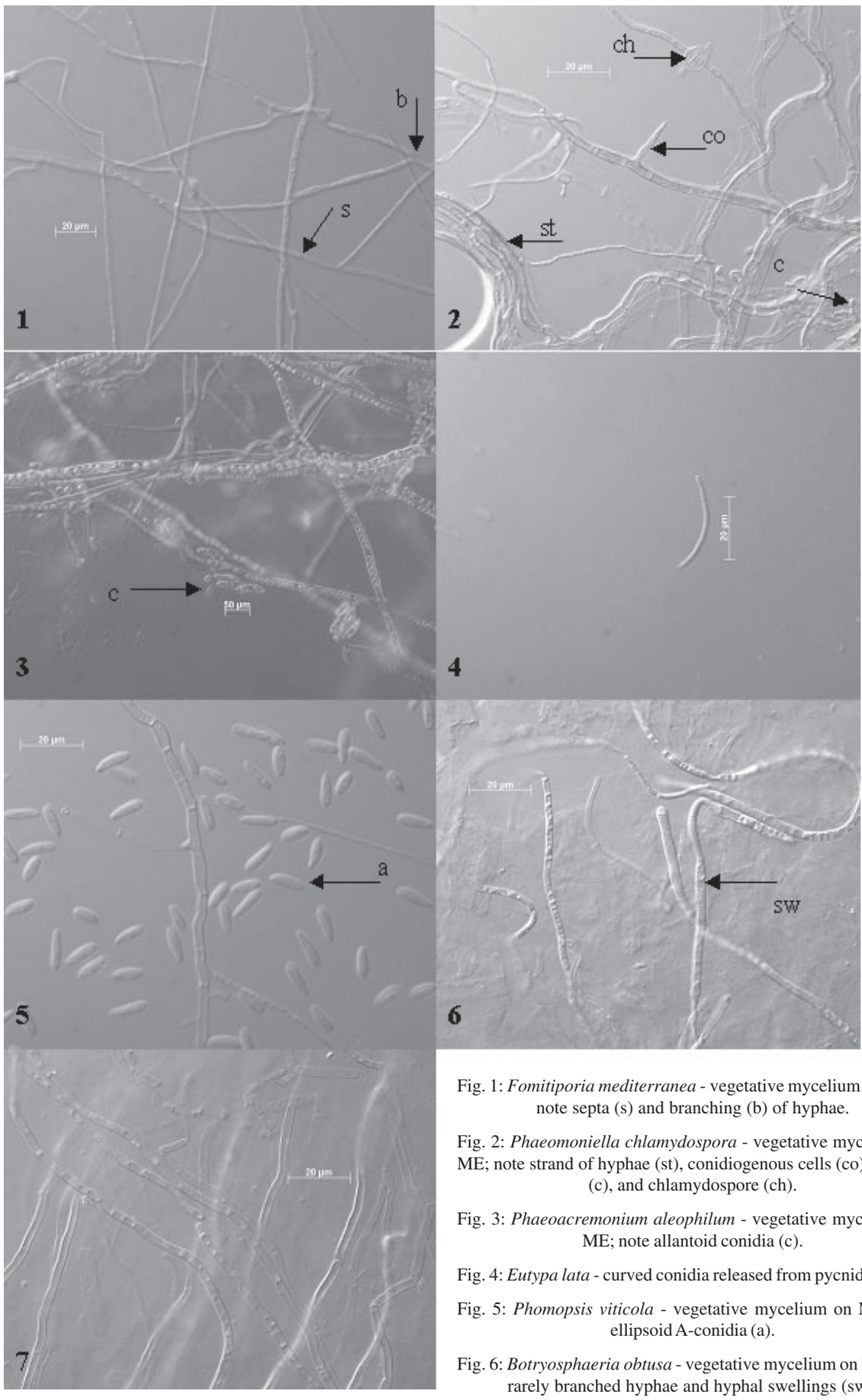

Fig. 1: *Fomitiporia mediterranea* - vegetative mycelium on ME; note septa (s) and branching (b) of hyphae.

Fig. 2: *Phaeomoniella chlamydospora* - vegetative mycelium on ME; note strand of hyphae (st), conidiogenous cells (co), conidia (c), and chlamydospore (ch).

Fig. 3: *Phaeoacremonium aleophilum* - vegetative mycelium on ME; note allantoid conidia (c).

Fig. 4: *Eutypa lata* - curved conidia released from pycnidium.

Fig. 5: *Phomopsis viticola* - vegetative mycelium on ME; note ellipsoid A-conidia (a).

Fig. 6: *Botryosphaeria obtusa* - vegetative mycelium on ME; note rarely branched hyphae and hyphal swellings (sw).

Fig. 7: *Cylindrocarpon destructans* - vegetative mycelium on ME; note rarely branched hyphae.

P. mortoniae. All these taxa are difficult to distinguish and their taxonomic rank may be not fully established yet. Further literature, providing data on morphology, microscopy, and molecular background: CROUS *et al*. (1996), DUPONT *et al*. (2000), and GROENEWALD *et al*. (2001).

4. *Eutypa lata* (Pers. : Fr.) Tul. & C. Tul.: Taxonomy: Member in Diatrypaceae, Diatrypales, Ascomycetes; anamorph *Libertella blepharis* A.L. Sm.

Cultured mycelium: Colonies cottony, white at first, becoming greyish to cream-coloured with age, reverse side becoming partly dark-grey to blackish, pigmentation of the medium concolorous, fast growing, 5.5-7.1 cm in 7 d; pycnidia formed after 3-4 weeks, black, scattered to slightly aggregated, up to 600 µm wide; conidial mass subglobose to globose, cream-colored to orange.

Microscopy (Fig. 4): Hyphae septate, branched, hyaline, smooth, length of hyphal segments very variable, without clamps, (1.0)1.5-4.0 (7.0) µm wide; ring-like structures rarely formed by single hyphae, up to 30 µm wide; conidia filiform, straight or curved, very numerous, 20.0-45.0 x 1.0-1.5 µm.

Nuclear behaviour: Hyphal segments uninucleate to binucleate; conidia uninucleate. ITS fragments (strain Ch.Mi.4): *Hpa II*: 5 fragments (22, 41, 76, 154, 261 bp); *Mbo I*: 3 fragments (3, 212, 339 bp). ITS sequence (strain Ch.Mi.4): 554 bp long.

AGGGATCATTACGGAGTTACCTAAACTCCAAACCCAT
GTGAACTTACTATGTTGCCTTGGGCGGGGAAGCTTAC
CCCGGTACTTACCTGATAGCTACCCGGGAGCGAGCTA
CCCTGTAGCCCGCTGCAGGCCTACCCGCCGGTGGACA
CTTAAACTCTTGTTTTTTAGTGATTATCTGAGTGTTTAT
ACTTAATAAGTTAAAACTTTCAACAACGGATCTCTTGG
TTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAA
GTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCT
TTGAACGCACATTGCGCCCATTAGTATTCTAGTGGGCAT
GCCTGTTCGAGCGTCATTTCGACCTTCAAGCCCTAGCTG
CTTGGTGTTGGGAGCCTATCTCCGGATAGCTCCTCAAAA
GCATTGGCGGAGTCGCGGTGGCCCCAAGCGTAGTAATTC
TTCGCGCTTTAGGTGTGTCACGGCTGACGTCTTGCCGTTA
AACCCCCAATTTTTTAAATGGTTGACCTCGGATAAGGTAG
GAATACCGCTGAACTTAA

Notes: The species forms perithecia on diseased trunks, and diversity of vegetative incompatibility types indicate a sexual reproduction by ascospores in nature (CORTESI *et al*. 2000 b). Quite remarkable, *E. lata* is frequently isolated from Esca-affected vines, partly side-by-side with *Fmed*. This may complicate the picture of Esca syndromes; however, *E. lata* is known to cause a distinct diesease, Eutypa dieback or Eutypiosis, with its own symptomatology. Besides, symptoms of Eutypiosis are evident for plants older than about 6 years only.

5. *Phomopsis viticola* (Sacc.) Sacc.: Taxonomy: Teleomorph in *Diaporthe* Nitschke, Diaporthales, Ascomycetes.

Cultured mycelium: Colonies wooly, slightly raised, sometimes with prominent growth rings, whitish at the beginning, then buff to honey to slightly pink, pigmentation of the medium concolorous, growth rates very variable, 2.2-5.0 cm in 14 d; pycnidia regularly formed on ME after 14 d, brown to black, scattered to aggregated, up to 500 µm wide, conidial mass more or less globose, pale yellow to dark grey.

Microscopy (Fig. 5): Hyphae septate, branched, hyaline to light brown, smooth, without clamps, (1.0) 1.5-5.0 µm wide; ring-like structures formed by single hyphae, 10-20 µm in diameter; A-conidia hyaline, unicellular, ellipsoid, (6.0) 7.0-10.0 x 2.0-3.0 (3.5) µm; B-conidia less common, hyaline, filiform, curved, 14.0-18.0 (22.0) x 1.0-2.0 µm.

Nuclear behaviour: Hyphal segments with 1-3 (7) nuclei; A-conidia uninucleate, B-conidia unknown. ITS fragments (strain MT.Zi.6): *Hpa* II: 7 fragments (10, 34, 70, 73, 86, 95, 192 bp); *Mbo* I: 3 fragments (38, 208, 314 bp). ITS sequence (strain MT.Zi.6): 560 bp long.

AACCCTTTGTGAACTCATACCTTACCGTTGCCTCGGC
GCAGGCCGGCCCCCCCCGGGGGGCCCCTCGGAGACG
AGGAGCAGGCCCGCCGGCGGCCAAGTTAACTCTTGTT
TTTACACTGAAACTCTGAGAAACAAAACACAAATGAA
TCAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATC
GATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAA
TTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCAC
ATTGCGCCCTCTGGTATTCCGGAGGGCATGCCTGTTCG
AGCGTCATTTCAACCCTCAAGCCTGGCTTGGTGATGGG
GCACTGCTCCCCCCCCCGGGGAGCAGGCCCTGAAATCC
AGTGGCGAGCTCGCCAGGACCCCGAGCGCAGTAGTTAA
ACCCTCGCTCCGGGAGGCCCTGGCGGTGCCCTGCCGTTA
AACCCCCAACTTCTGAAAGTT

Notes: *Phomopsis viticola* is another fungus detected quite regularly in Esca-affected vine. However, the diseases caused by *P. viticola* are distinct from Esca, and are known as "black arm disease" or "cane and leaf spot disease". It should be noted that taxonomy and pathogenicity within *P. viticola* has not been fully clarified yet. A comprehensive overview is provided by MOSTERT *et al*. (2001).

6. *Botryosphaeria obtusa* (Schwein.) Shoemaker: Taxonomy: Teleomorph not formed in artificial culture; member in Botryosphaeriaceae, Pleosporales, Asomycetes; anamorph within *Diplodia* Fr. apud Mont.

Cultured mycelium: Colonies wooly, with dense aerial mycelium, grey-brown to olivaceous-brown, pigmentation of the medium concolorous, fast growing, 4.8-6.2 cm in 4 d, no pycnidia formed on ME (see "Notes" below).

Microscopy (Fig. 6): Hyphae septate, rarely branched, hyaline to light brown, smooth, without clamps, (1.5) 2.5-5.0 (14.0) µm wide; hyphal swellings present in some cultures, single or in chains of up to 5, 6.0-10.0 x 3.5-5.0 µm; conidia absent on ME.

Nuclear behaviour: Hyphal segments oligonucleate, with (1-) 2-6 (-10) nuclei. ITS fragments (strain We.Oc.2): *Hpa* II: 3 fragments (56, 160, 319 bp); *Mbo* I: 4 fragments (8, 18, 197, 312 bp). ITS sequence (strain We.Oc.2): 535 bp long.

AAGGATCATTACCGAGTTCTCGGGCTTCGGCTCGAAT
CTCCCACCCTTTGTGAACATACCTCTGTTGCTTTGGCG
GCTCTTTGCCGCGAGGAGGCCCTCGCGGGCCCCCCCG
CGCGCTTTCCGCCAGAGGACCTTCAAACTCCAGTCAG
TAAACGTCGACGTCTGATAAACAAGTTAATAAACTAA

AACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATG
AAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGC
AGAATTCAGTGAATCATCGAATCTTTGAACGCACATTG
CGCCCCCTGGCATTCCGGGGGGCATGCCTGTTCGAGCG
TCATTACAACCCTCAAGCTCTGCTTGGTATTGGGCGCC
GTCCTCTCTGCGGACGCGCCTTAAAGACCTCGGCGGTG
GCTGTTCAGCCCTCAAGCGTAGTAGAATACACCTCGCT
TTGGAGCGGTTGGCGTCGCCCGCCGGACGAACCTTCTG
AACTTTTCTCAAGGTTGACCTCGGATCAGGTAGGGATA
CCC

N o t e s : Species of *Botryosphaeria* cause canker and dieback of pomaceous fruits and grapevine. In Bordeaux vineyards, *B. obtusa* was found to be associated with black dead arm of grapevine (Larignon and Dubos 2001), showing similar foliar symptoms to those caused by Esca. While *B. obtusa* is not considered to be associated with Esca, it can be sometimes isolated from affected vines. According to Phillips (2002), pycnidia are formed after several days on oatmeal agar; they are scattered over the agar surface, up to 1 mm in diameter; conidia are cylindric, hyaline when young, becoming dark brown when old, smooth, (10.0) 15.0-25.0 x (7.0) 10.0-13.0 µm.

7. *C y l i n d r o c a r p o n d e s t r u c t a n s* (Z i n s .) S c h o l t e n : T a x o n o m y : Deuteromyces (Fungi imperfecti); teleomorph *Nectria radicicola* Gerlach & Nilsson, Hypocreaceae, Sphaeriales, Ascomycetes.

C u l t u r e d m y c e l i u m : Colonies cottonny to fluffy, appressed at the margin, white to buff, sometimes with prominent growth rings, these with appressed mycelium, reverse side concolorous, growth rate 2.8-3.7 cm in 14 d.

M i c r o s c o p y (Fig. 7): Hyphae septate, rarely branched, sometimes forming strands of up to 10, mostly straight, hyaline, smooth, without clamps, (1.5) 2.0-3.0 (6.0) µm wide; hyphal swellings present in most cultures, intercalar, in chains of up to 20, up to 10.0 µm in diameter; conidia absent.

N u c l e a r b e h a v i o u r : Hyphal segments with (1) 2-8 nuclei. ITS fragments (strain MT.Zi.Z3): *Hpa* II: 4 fragments (10, 32, 146, 341 bp); *Mbo* I: 5 fragments (27, 29, 143, 156, 174 bp). ITS sequence (strain MT.Zi.Z3): 529 bp long.

AGTTATCCGTTGGGGAACCAGCGGAGGGATCATTACC
GAGTTTACAACTCCCAAACCCCTGTGAACATACCATTT
GTTGCCTCGGCGGTGCCTGCTTCGGCAGCCCGCCAGAG
GACCCAAACCCTTGATTTTATACAGTATCTTCTGAGTA
AATGATTAAATAAATCAAAACTTTCAACAACGGATCTC
TTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGA
TAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAA
TCTTTGAACGCACATTGCGCCCGCCAGTATTCTGGCGG
GCATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCCCC
CGGGCTTGGTGTTGGAGATCGGCGTGCCCCCCCGGGGC
GCGCCGGCTCCCAAATATAGTGGCGGGCTCGCTGTAGC
TTCCTCTGCGTAGTAGCACACCTCGCACTGGAAAACAG
CGTGGCCACGCCGTTAAACCCCCCACTTCTGAAAGGTT
GACCTCGGATCAGGTAGGAATACCCGCTGAACTTAA

N o t e s : In Portugal, *C. destructans* along with species of *Phaeoacremonium* is the fungus most frequently isolated out of discoloured wood (Rego *et al*. 2000) and is thought to be involved in young vine decline or Petri disease. The fungus has been reported to be the causal agent of black-foot disease in Italy and France. *Cylindrocarpon destructans* has only rarely been observed in our study.

T a b l e 2

Lignicolous fungi on grapevine

Species (literature)	Life strategy	Type of rot	Spread
Armillaria mellea (Kreisel 1961)	saprophytic, parasitic	white rot	rhizomorphs, spores
Clitopilus hobsonii (Fischer unpubl.)	saprophytic	unknown	spores
Flammulina velutipes (Kreisel 1961)	parasitic	white rot	spores
Pleurotus pulmonarius (Fischer unpubl.)	saprophytic, parasitic	white rot	spores
Inonotus hispidus (Ryvarden and Gilbertson 1993)	parasitic	white rot	spores
Stereum hirsutum (Mugnai et al. 1996)	saprophytic, (parasitic)	white rot	spores
Trametes hirsuta (Fischer unpubl.)	saprophytic, (parasitic)	white rot	spores
Trametes versicolor (Fischer unpubl.)	saprophytic, (parasitic)	white rot	spores
Peniophora incarnata (Fischer unpubl.)	saprophytic	white rot?	spores
Hirneola auriculae-judae (Fischer unpubl.)	saprophytic, parasitic	white rot?	spores

Appendix - occurrence of basidiomycetous fruitbodies on grapevine: Wood-inhabiting fungi are able to utilize components of wood cell walls as their main source of energy for growth and reproduction. They can be grouped into two categories depending on the enzyme system they produce to decay wood. Grapevine, as most other hardwoods, is susceptible to white rot fungi, decomposing both lignin and polysaccharides. Therefore, it is not surprising that a considerable number of white rot basidiomycetes can be found on grapevine. The fruitbodies of basidiomycetous taxa that have been detected on vine in the geographic area under study in 2001 and 2002 were identified (Tab. 2). For all taxa, some basal information is provided with respect to life strategy, i.e. saprophytes and/or parasites, type of rot, and mode of spread. While *Armillaria mellea*, *Flammulina velutipes*, *Inonotus hispidus*, and *Stereum hirsutum* have been known before as occurring on *Vitis vinifera*, several taxa, namely *Clitopilus hobsonii*, *Pleurotus pulmonarius*, *Trametes hirsuta*, *T. versicolor*, *Peniophora incarnata*, and *Hirneola auricula-judae* are demonstrated here for the first time as living on grapevine. All species in Tab. 2 are not restricted to *Vitis*, and can be found on a considerable number of other hardwood genera as well. Certainly, additional vine-inhabiting fungi will be detected in the future.

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